DESCRIPTION

METHOD OF PREDICTING GENETIC RISK FOR HYPERTENSION

TECHNICAL FIELD

The present invention relates to a detection method using genes associated with hypertension. More particularly, it relates to a detection method using a plurality of gene polymorphisms associated with hypertension and to a kit used for the method. The present invention can be used for diagnosing a risk of development of hypertension.

BACKGROUND ART

Hypertension is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (see non-patent document 1). Given that hypertension is a major risk factor for coronary artery disease, stroke, and chronic renal failure, prevention of hypertension is an important public health goal. One approach to preventing the development of hypertension is to identify susceptibility genes. Linkage studies (see non-patent documents 2 to 4) and association studies with candictae genes (see non-patent documents 5 to 8) have implicated various chromosomal loci and genes in predisposition to hypertension. Although genetic epidemiological studies have suggested that certain genetic variants, including polymorphisms in the genes encoding angiotensinogen (non-patent document 5), α-adducin (non-patent document 6), the β3 subunit of G proteins (non-patent document 7) and the β2-adrenergic receptor (non-patent document 8), etc. increase the risk for hypertension, the genes that contribute to genetic susceptibility to hypertension remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms, it is important to construct a database of polymorphisms related to hypertension in each ethnic group.

Non-patent document 1: Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell. 2001;104: 545-556.

Non-patent document 2: Xu X, Rogus JJ, Terwedow HA, Yang J, Wang Z, Chen C, Niu T, Wang B, Xu H, Weiss S, Schork NJ, Fang Z. An extreme-sib-pair genome scan for genes regulating blood pressure. Am J Hum Genet. 1999;64: 1694-1701.

Non-patent document 3: Krushkal J, Ferrell R, Mockrin SC, Turner ST, Sing CF, Boerwinkle E. Genome-wide linkage analysis of systolic blood pressure using highly discordant siblings. Circulation. 1999;99: 1407-141.

Non-patent document 4: Rice T, Rankinen T, Province MA, Chagnon YC, Pérusse L, Borecki IB, Bouchard C, Rao DC. Genome-wide linkage analysis of

systolic and diastolic blood pressure: the Québec Family Study. Circulation. 2000;102: 1956-1963.

Non-patent document 5: Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel J-M, Corvol P. Molecular basis of human hypertension: role of angiotensinogen. Cell. 1992;71: 169-180.

Non-patent document 6: Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, Gloriso N, Lanzani C, Manunta P, Righetti M, Rivera R, Stella P, Troffa C, Zagato L, Bianchi G. Polymorphisms of a-adducin and salt sensitivity in patients with essential hypertension. Lancet. 1997;349: 1353-1357.

Non-patent document 7: Siffert W, Rosskop D, Siffert G, Busch S, Moritz A, Erbel R, Sharma AM, Ritz E, Wichmann H-E, Jakobs KH, Horsthemke B. Association of a human G-protein ß3 subunit variant with hypertension. Nat Genet. 1998;18: 45-48.

Non-patent document 8: Bray MS, Krushkal J, Li L, Ferrell R, Kardia S, Sing CF, Turner ST, Boerwinkle E. Positional genomic analysis identifies the ß2-adrenergic receptor gene as a susceptibility locus for hypertension. Circulation. 2000;101: 2877-2882.

SUMMARY OF THE INVENTION

As mentioned above, many association studies have previously examined the relations between gene polymorphisms and hypertension. The results of most of these studies, however, remain controversial, with no consensus on their implications, mainly because of the limited population size of the studies, the ethnic diversity of gene polymorphisms, and complicating environmental factors. Furthermore, even though associations with respect to hypertension have been detected, the relative risk (odds ratio) has tended to be low in large populations.

The present invention was made on the basis of the above-mentioned background, and the object thereof is to provide a means of diagnosing genetic risk for hypertension with high accuracy and high predictability so as to contribute primary prevention of hypertension.

To achieve the above-mentioned objects, the present inventors have extracted 71 genes which were estimated to be associated with coronary arteriosclerosis, coronary artery spasm, hypertension, diabetes mellitus, hyperlipidemia, etc., and mainly selected 112 polymorphisms which were predicted to be associated with functional changes of genes by the use of a plurality of public databases. Then, as to 112 polymorphisms of 71 genes, association study with respect to myocardial infarction was carried out in 445 myocardial cases and 464 controls. As a result, the present inventors have identified 19 and 18 single nucleotide polymorphisms (SNPs)

related to myocardial infarction in men and women, respectively (Yamada Y, Izawa H, Ichihara S, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med. in press). However, these SNPs also include candidate determinants of the susceptibility to hypertension. Then, the present inventors therefore performed a large-scale association study for these SNPs and hypertension. As a result, the present inventors succeeded in identifying four and four SNPs related to hypertension in men and women, respectively. In addition, analysis of the combination of these polymorphisms revealed maximal odds ratios of 5.34 for men and 46.86 for women, respectively, on the basis of the stepwise forward selection method. In the analysis, the odds ratios were maximum among the odds ratios which had been reported in the past. Based on these results, it was possible to obtain findings that by selecting a plurality of SNPs from these SNPs and using the combination of the results of analysis of each SNP, diagnosis of hypertension can be carried out with high reliability and high predictability. The present invention was made based on the above-mentioned findings and provides the following configuration.

[1] A method for detecting the genotype in a nucleic acid sample, the method comprising the following step (a):

(a) analyzing two or more polymorphisms selected from the group consisting of the following (1) to (4) in a nucleic acid sample:

(1) a polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(2) a polymorphism at the base number position 190 of the chemokine receptor 2 gene;

(3) a polymorphism at the base number position 1100 of the apolipoprotein C-III gene; and

(4) a polymorphism at the base number position 825 of G-protein β3 subunit gene.

[2] A method for detecting the genotype in a nucleic acid sample, the method comprising the following step (b):

(b) analyzing two or more polymorphisms selected from the group consisting of the following (5) to (8) in a nucleic acid sample:

(5) a polymorphism at the base number position -850 of the tumor necrosis factor-α gene;

(6) a polymorphism at the base number position -238 of the tumor necrosis factor-α gene;

(7) a polymorphism at the base number position 3494 of the insulin receptor substrate-1 gene; and

(8) a polymorphism at the base number position 1018 of the glycoprotein Ibα

gene.

[3] A method for diagnosing the risk for hypertension, comprising the following steps (i) to (iii):

(i) analyzing two or more polymorphisms selected from the group consisting of the following (1) to (4) in a nucleic acid sample:

(1) a polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(2) a polymorphism at the base number position 190 of the chemokine receptor 2 gene;

(3) a polymorphism at the base number position 1100 of the apolipoprotein C-III gene; and

(4) a polymorphism at the base number position 825 of G-protein β3 subunit gene.

(ii) determining, based on the information about polymorphism which was obtained in the step (i), the genotype in the nucleic acid sample; and

(iii) assessing, based on the genotype determined, a genetic risk for hypertension.

[4] A method for diagnosing the risk for hypertension, comprising the following steps (iv) to (vi):

(iv) analyzing two or more polymorphisms selected from the group consisting of the following (5) to (8) in a nucleic acid sample:

(5) a polymorphism at the base number position -850 of the tumor necrosis factor-α gene;

(6) a polymorphism at the base number position -238 of the tumor necrosis factor-α gene;

(7) a polymorphism at the base number position 3494 of the insulin receptor substrate-1 gene; and

(8) a polymorphism at the base number position 1018 of the glycoprotein Ibα gene.

(v) determining, based on the information about polymorphism which was obtained in the step (iv), the genotype in the nucleic acid sample; and

(vi) assessing, based on the genotype determined, a genetic risk for hypertension.

[5] A kit for detecting the genotype, comprising two or more of nucleic acids selected from the group consisting of the following (1) to (4):

(1) a nucleic acid for analyzing a polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(2) a nucleic acid for analyzing a polymorphism at the base number position 190 of the chemokine receptor 2 gene;

(3) a nucleic acid for analyzing a polymorphism at the base number position 1100 of the apolipoprotein C-III gene; and

(4) a nucleic acid for analyzing a polymorphism at the base number position 825 of G-protein β3 subunit gene.

[6] A kit for detecting the genotype, comprising two or more of nucleic acids selected from the group consisting of the following (5) to (8):

(5) a nucleic acid for analyzing a polymorphism at the base number position -850 of the tumor necrosis factor-α gene;

(6) a nucleic acid for analyzing a polymorphism at the base number position -238 of the tumor necrosis factor-α gene;

(7) a nucleic acid for analyzing a polymorphism at the base number position 3494 of the insulin receptor substrate-1 gene; and

(8) a nucleic acid for analyzing a polymorphism at the base number position 1018 of the glycoprotein Ibα gene.

[7] Fixed nucleic acids comprising the following two or more nucleic acids selected from the group consisting of the following (1) to (4) fixed to an insoluble support:

(1) a nucleic acid for analyzing a polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(2) a nucleic acid for analyzing a polymorphism at the base number position 190 of the chemokine receptor 2 gene;

(3) a nucleic acid for analyzing a polymorphism at the base number position 1100 of the apolipoprotein C-III gene; and

(4) a nucleic acid for analyzing a polymorphism at the base number position 825 of G-protein β3 subunit gene.

[8] Fixed nucleic acids comprising the following two or more nucleic acids selected from the group consisting of the following (5) to (8) fixed to an insoluble support:

(5) a nucleic acid for analyzing a polymorphism at the base number position -850 of the tumor necrosis factor-α gene;

(6) a nucleic acid for analyzing a polymorphism at the base number position -238 of the tumor necrosis factor-α gene;

(7) a nucleic acid for analyzing a polymorphism at the base number position 3494 of the insulin receptor substrate-1 gene; and

(8) a nucleic acid for analyzing a polymorphism at the base number position 1018 of the glycoprotein Ibα gene.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a table summarizing 112 gene polymorphisms examined in a screening association study in Examples.

Figure 2 is also a table summarizing 112 gene polymorphisms examined in a screening association study in Examples.

Figure 3 is a table summarizing primers (SEQ ID NOs: 16, 17, 18, 14, 15, 11, 12, 13, 8, 9, 10, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30 in this order from the top), probes (SEQ ID NOs: 31, 32, 33 and 34 in this order from the top) and other conditions used to determine the genotype in Examples. In Figure 3, FITC denotes fluorescein isothiocyanate, TxR denotes Texas Red and Biotin denotes biotin, respectively.

Figure 4 is a table summarizing single nucleotide polymorphisms examined in an association study in Examples.

Figure 5 is a table summarizing the background data of 1107 lesions in men and 833 lesions in women examined in an association study in Examples. Each data of age, body mass index, systolic blood pressure, diastolic blood pressure, and serum creatinine is represented by means ± standard deviation. In table, *1 denotes $P < 0.0001$, *2 denotes $P < 0.001$, and *3 denotes $P < 0.01$, respectively.

Figure 6 is a table summarizing gene polymorphisms and results of multivariate logistic regression analysis examined in the association study. In each SNP, smaller P value is expressed in boldface.

Figure 7 is a view showing a distribution of genotype of gene polymorphisms associated with hypertension.

Figure 8 is a table showing results of stepwise forward selection method of multivariate logistic regression analysis of gene polymorphisms associated with hypertension.

Figure 9 is a table showing results of diagnosis of genetic risk for hypertension using a combination of four gene polymorphisms in men.

Figure 10 is a table showing results of diagnosis of genetic risk for hypertension using a combination of four gene polymorphisms in women.

Figure 11 is a graph showing a correlation between the cumulative odds ratio for hypertension and the number of single nucleotide polymorphisms. (A) shows the correlation in men and (B) shows the correlation in women. In (A), SNPs include: SNP1: GPIa (1648A→G) polymorphism, SNP 2: CCR2 (190G→A) polymorphism, SNP3: ApoC-III (1100C→T) polymorphism, and SNP4: GPß3 (825C→T) polymorphism. In (B), SNPs include: SNP1: TNFa (-850C→T) polymorphism, SNP2: TNFa (-238G→A) polymorphism, SNP3: IRS-1 (3494G→A) polymorphism, and SNP4: GPIba (1018C→T) polymorphism.

BEST MODE FOR CARRYING OUT THE INVENTION

The first aspect of the present invention relates to a method of detecting the genotype in a nucleic acid sample. One embodiment of the present invention is featured by including the step of analyzing two or more polymorphisms selected from the group consisting of the following (1) to (4). Another embodiment is featured by including the step of analyzing two or more polymorphisms selected from the group consisting of the following (5) to (8). Note here that it is possible to determine, based on the information about polymorphisms which was obtained in the above-mentioned step, the genotype in the nucleic acid sample, and thereby to assess, based on the genotype determined, a genetic risk for hypertension.

(1) a polymorphism at the base number position 1648 of the glycoprotein Ia gene: 1648A→G (hereinafter, also referred to as "GPIa (1648A→G) polymorphism")

(2) a polymorphism at the base number position 190 of the chemokine receptor 2 gene: 190G→A (hereinafter, also referred to as "CCR2 (190G→A) polymorphism")

(3) a polymorphism at the base number position 1100 of the apolipoprotein C-III gene: 1100C→T (hereinafter, also referred to as "ApoC-III (1100C→T) polymorphism")

(4) a polymorphism at the base number position 825 of G-protein β3 subunit gene: 825C→T (hereinafter, also referred to as "GPβ3 (825C→T) polymorphism")

(5) a polymorphism at the base number position -850 of the tumor necrosis factor-α gene: -850C→T (hereinafter, also referred to as "TNFa (-850C→T) polymorphism")

(6) a polymorphism at the base number position -238 of the tumor necrosis factor-α gene: -238G→A (hereinafter, also referred to as "TNFa (-238G→A) polymorphism")

(7) a polymorphism at the base number position 3494 of the insulin receptor substrate-1 gene: 3494G→A (hereinafter, also referred to as "IRS-1 (3494G→A) polymorphism")

(8) a polymorphism at the base number position 1018 of the glycoprotein Ibα gene: 1018C→T (hereinafter, also referred to as "GPIba (1018C→T) polymorphism")

In the above, description such as 1648A→G means that polymorphism at the relevant base number position consists of two genotypes, bases before and after the arrow.

The base number of each gene is expressed using as standards the known sequences which are registered in the public database, GenBank (NCBI). Note here that in the base sequence of SEQ ID NO: 1 (Accession No.X17033 M28249: Human mRNA for integrin alpha-2 subunit), the 1648th base corresponds to the base at position 1648 of the glycoprotein Ia gene. Similarly, in the base sequence of SEQ ID

NO: 2 (Accession No. U95626: Homo sapiens ccr2b (ccr2), ccr2a (ccr2), ccr5 (ccr5) and ccr6(ccr6) genes, complete cds, and lactoferrin (lactoferrin) gene, partial cds, complete sequence (wherein, sequence of SEQ ID NO: 2 is a sequence to 50,000th base sequence)), the 46295th base corresponds to the base at position 190 of the chemokine receptor 2 gene; in the base sequence of SEQ ID NO: 3 (Accession No. X01392: Human apolipoprotein CIII gene and apo AI-apo CIII intergenic), the 1100th base corresponds to the base at position 1100 of the apolipoprotein C-III gene; in the base sequence of SEQ ID NO: 4 (Accession No. M31328: Human guanine nucleotide-binding protein beta-3 subunit mRNA, complete cds.), the 831st base corresponds to the base at position 825 of the G-protein β3 subunit gene; in the base sequence of SEQ ID NO: 5 (Accession No. L11698: Homo sapiens tumor necrosis factor alpha gene, promoter region.), the 203rd base corresponds to the base at position -850 of the tumor necrosis factor a gene; in the sequence of SEQ ID NO: 5 (Accession No. L11698: Homo sapiens tumor necrosis factor alpha gene, promoter region.), the 816th base corresponds to the base at position -238 of the tumor necrosis factor a gene; in the sequence of SEQ ID NO: 6 (Accession No. S85963: hIRS-1=rat insulin receptor substrate-1 homolog [human, cell line FOCUS, Genomic, 6152 nt]), the 3494th base corresponds to the base at position 3494 of the insulin receptor substrate-1 gene; and in the sequence of SEQ ID NO: 7 (Accession No. J02940: Human platelet glycoprotein Ib alpha chain mRNA, complete cds.), the 524th base corresponds to the base at position 1018 of the glycoprotein Iba gene.

In the present invention, "analyzing polymorphism" means the investigation as to what genotype a nucleic acid sample has in the gene polymorphism to be analyzed. It is the same meaning as the investigation on the base (base sequence) of the position in which the polymorphism exists. Typically, for example, in the case of the analysis of the GPIa (1648A→G) polymorphism, it refers to investigation on what genotype, i.e., AA (the base at position 1648 is a homozygote of allele A), AG (the base at position 1648 is a heterozygote of allele A and allele G) and GG (the base at position 1648 is a homozygote of allele G), the glycoprotein Ia gene in a nucleic acid sample has.

As shown in Examples mentioned below, the polymorphisms mentioned (1) to (4) above are polymorphisms that are recognized as being particularly effective to be used in determining genetic risk for hypertension in an analysis of Japanese male subjects. Therefore, analysis targeting these polymorphisms enables diagnosis with higher accuracy and with higher predictability when subjects are men (particularly, Japanese men).

Similarly, as shown in Examples mentioned below, the polymorphisms

mentioned (5) to (8) above are polymorphisms that are recognized as being particularly effective to be used in determining genetic risk for hypertension in an analysis of Japanese female subjects. Therefore, analysis targeting these polymorphisms enables diagnosis with higher accuracy and with higher predictability when subjects are women (particularly, Japanese women).

Herein, in principle, in proportion to the increase in the number of polymorphisms to be analyzed, the genotypes of nucleic acid sample are classified more finely. Thereby, it is possible to diagnose a genetic risk for hypertension with higher predictability. From this viewpoint, it is preferable to detect the genotype by analyzing a larger number of polymorphisms in the above-mentioned polymorphisms (1) to (4). Therefore, it is the most preferable to analyze all of the polymorphisms (1) to (4). In the case where detection is carried out by combining three or less of polymorphisms, it is preferable to preferentially select the polymorphisms with higher odds ratios as in Examples mentioned below. For example, in the case where three polymorphisms are used in combination, it is preferable to select three polymorphisms with higher odds ratio, that is, to select (2), (3) and (4). Similarly, in the case where two polymorphisms are used in combination, it is preferable to select (2) and (4).

Similarly, in the case where two or more polymorphisms selected from the group consisting of polymorphisms (5) to (8), it is most preferable to analyze all these polymorphisms, that is, four polymorphisms. In the case where detection is carried out by combining three or less of polymorphisms, it is preferable to preferentially select the polymorphisms with higher odds ratios in Examples mentioned below. For example, in the case where three polymorphisms are used in combination, it is preferable to select (5), (7) and (8). Similarly, in the case where two polymorphisms are used in combination, it is preferable to select (5) and (7).

A method for analyzing each genetic polymorphism is not particularly limited and known method can be employed. The known methods may include, for example, amplification by PCR using an allele-specific primer (and probe), a method for analyzing polymorphism of amplified product by means of fluorescence or luminescence; a method using a PCR (polymerase chain reaction) method including a PCR-RFLP (polymerase chain reaction–restriction fragment length polymorphism) method, a PCR-SSCP (polymerase chain reaction–single strand conformation polymorphism) method (Orita, M. et al., Proc. Natl. Acad. Sci., U.S.A., 86, 2766-2770 (1989), etc.), and a PCR-SSO (specific sequence oligonucleotide) method, an ASO (allele specific oligonucleotide) hybridization method combining the PCR-SSO method and a dot hybridization method (Saiki, Nature, 324, 163-166 (1986), etc.), or a TaqMan-PCR method (Livak, KJ, Genet Anal, 14, 143 (1999), Morris, T. et al., J. Clin.

Microbiol.,34, 2933 (1996)), an Invader method (Lyamichev V et al., Nat Biotechnol, 17, 292 (1999)), a MALDI-TOF/MS (matrix) method using a primer extension method (Haff LA, Smirnov IP, Genome Res 7, 378 (1997)), a RCA (rolling cycle amplification) method (Lizardi PM et al., Nat Genet 19,225 (1998)), a method using DNA microchip or micro-array (Wang DG et al., Science 280, 1077 (1998), etc.)), a primer extension method, a Southern blot hybridization method, a dot hybridization method (Southern, E., J. Mol. Biol. 98, 503-517 (1975)), etc.), or the like. Furthermore, an analysis may be made by direct sequencing of the portion of polymorphism which is subject to analysis. Note here that polymorphisms may be analyzed by combining these methods ad libitum.

In the case where the amount of nucleic acid sample is small, it is preferable to analyze it by a method using PCR (for example, PCR-RFLP method) from the viewpoint of detection sensitivity or accuracy. Furthermore, any of the above-mentioned analysis methods may be employed after nucleic acid sample is amplified in advance (including a partial region of nucleic acid sample) by a gene amplification such as PCR method or a method applying PCR method.

Meanwhile, in the case where a large number of nucleic acid samples are analyzed, it is particularly preferable to employ a method capable of analyzing a large number of samples in a relatively short period of time, for example, allele-specific PCR method, allele-specific hybridization method, TaqMan-PCR method, Invader method, MALDI-TOF/MS (matrix) method using a primary extension method, RCA (rolling cycle amplification) method, a method using a DNA chip or a micro-array, or the like.

The above methods use nucleic acids (also called "nucleic acid for analyzing polymorphism" in the present invention), e.g., primer and probe, in accordance with each method. An example of the nucleic acids for analyzing polymorphism may include a nucleic acid having a sequence complementary to a given region including the site of polymorphism (partial DNA region) in a gene which contains polymorphism to be analyzed, and a nucleic acid (primer) which has a sequence complementary to a given region including the site of polymorphism (partial DNA region) in a gene which contains polymorphism to be analyzed and which is designed to specifically amplify the DNA fragment containing the relevant site of polymorphism. In the case where polymorphism at position 1648 of the glycoprotein Ia gene is a subject to be analyzed, an example of such nucleic acids includes a nucleic acid having a sequence complementary to a partial DNA region including the position 1648 of the glycoprotein Ia gene whose base at position 1648 is A (adenine), or a nucleic acid having a sequence complementary to a partial DNA region including the position 1648 of the glycoprotein Ia gene whose base at position 1648 is G (guanine).

Other concrete examples of nucleic acids for analyzing polymorphism may include a set of nucleic acids which is designed to specifically amplify the partial DNA region that contains the relevant site of polymorphism only in the case where the site of polymorphism to be analyzed is a certain genotype. A more concrete example may include a set of nucleic acids which is designed to specifically amplify the partial DNA region including the site of polymorphism to be analyzed and which consists of a sense primer that specifically hybridizes the partial DNA region including the relevant site of polymorphism in an antisense strand whose site of polymorphism is a certain genotype and of an antisense primer that specifically hybridizes a partial region in the sense strand. In the case where a subject to the analysis is a polymorphism at position 1648 of the glycoprotein Ia gene, examples of such a set of nucleic acids include a set of nucleic acids which is designed to specifically amplify the partial DNA region including the base at position 1648 of the glycoprotein Ia gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1648 in the antisense strand of the glycoprotein Ia gene whose base at position 1648 is A (adenine) and of an antisense primer that specifically hybridizes a partial region in the sense strand; or a set of nucleic acids which consists of a sense primer that specifically hybridizes the partial DNA region including the base at position 1648 in the antisense strand of the glycoprotein Ia gene whose base at position 1648 is G (guanine) and of an antisense primer that specifically hybridizes a partial region in the sense strand. The length of the partial DNA region to be amplified here is set accordingly in a range which is appropriate for its detection, and is for example, 50 bp to 200 bp, and preferably 80 bp to 150 bp. A more concrete example may include a set of nucleic acid primers for analyzing the GPIa (1648A→G) polymorphism containing the following sequences. Note here that an underlined part in the following sequences means a part corresponding to the polymorphism. Furthermore, in the sequence, N denotes any of A, T, C and G.

sense primer

GAGTCTACCTGTTTACTATCAANAA: SEQ ID NO: 8, or

GAGTCTACCTGTTTACTATCAANGA: SEQ ID NO: 9

antisense primer

ACCAGTACTAAAGCAAATTAAACT: SEQ ID NO: 10

Similarly, an example of a nucleic acid primer for analyzing the CCR2 (190G→A) polymorphism may include a set containing the following sequences.

antisense primer

GCAGTTTATTAAGATGAGGNCG: SEQ ID NO: 11, or

TTGCAGTTTATTAAGATGAGGNTG: SEQ ID NO: 12

sense primer
GGTGCTCCCTGTCATAAATTTGA: SEQ ID NO: 13

Similarly, an example of a nucleic acid primer for analyzing the ApoC-III (1100C→T) polymorphism may include a set containing the following sequences.
sense primer
CCTTCTCAGCTTCATGCAGG: SEQ ID NO: 14,
antisense primer
GTCTTGGTGGCGTGCTTCA: SEQ ID NO: 15

Similarly, an example of a nucleic acid primer for analyzing the GPß3 (825C→T) polymorphism may include a set containing the following sequences.
sense primer
TCTGCGGCATCACGTNCG: SEQ ID NO: 16, or
TCTGCGGCATCACGTNTG: SEQ ID NO: 17
antisense primer
GAATAGTAGGCGGCCACTGA: SEQ ID NO: 18

Similarly, an example of a nucleic acid primer for analyzing the TNFa (-850C→T) polymorphism may include a set containing the following sequences.
antisense primer
TCTACATGGCCCTGTCTTNGT: SEQ ID NO: 19, or
CTCTACATGGCCCTGTCTTNAT: SEQ ID NO: 20
sense primer
CTCTACATGGCCCTGTCTTTAT: SEQ ID NO: 21

Similarly, an example of a nucleic acid primer for analyzing the TNFa (-238G→A) polymorphism may include a set containing the following sequences.
antisense primer
CCCCATCCTCCCTGCTNCG: SEQ ID NO: 22, or
CCCCATCCTCCCTGCTNTG: SEQ ID NO: 23
sense primer
AGTCAGTGGCCCAGAAGACC: SEQ ID NO: 24

Similarly, an example of a nucleic acid primer for analyzing the IRS-1 (3494G→A) polymorphism may include a set containing the following sequences.
sense primer
GGGCCCTGCACCTCCNGG: SEQ ID NO: 25, or
GGGCCCTGCACCTCCNAG: SEQ ID NO: 26
antisense primer

GGGTAGGCCTGCAAATGCTA: SEQ ID NO: 27

Similarly, an example of a nucleic acid primer for analyzing the GPIba (1018C→T) polymorphism may include a set containing the following sequences.

sense primer

CCCAGGGCTCCTGNCG: SEQ ID NO: 28, or

CCCCAGGGCTCCTGNTG: SEQ ID NO: 29

antisense primer

TGAGCTTCTCCAGCTTGGGTG: SEQ ID NO: 30

On the other hand, a concrete example of the probe can include:

as a probe for analyzing ApoC-III (1100C→T) polymorphism,

CAGCTTCATGCAGGGCTACA: SEQ ID NO: 31, or

CAGCTTCATGCAGGGTTACA: SEQ ID NO: 32

as a probe for analyzing TNFa (-850C→T) polymorphism,

ACATGGCCCTGTCTTNGTTAAG: SEQ ID NO: 33, or

ACATGGCCCTGTCTTNATTAAG: SEQ ID NO: 34

as a probe for analyzing IRS-1 (3494G→A) polymorphism,

CACCTCCNGGGGCTGCTAG: SEQ ID NO: 35, or

CACCTCCNAGGGCTGCTAG: SEQ ID NO: 36

The above nucleic acid primers and nucleic acid probes are mere examples. Nucleic acid primers may contain a partially modified base sequence as long as they can carry out the aimed amplification reaction without inconvenience, while nucleic acid probes may contain a partially modified base sequence as long as they can carry out the aimed hybridization reaction without inconvenience. "Partially modified" herein means that a part of bases is deleted, replaced, inserted, and/or added. The numbers of bases to be modified are, for example, one to seven, preferably one to five, and more preferably one to three. Note here that such a modification is made in the portions other than bases corresponding to the site of polymorphism, in principle.

As nucleic acids (probes or primers) for analyzing polymorphism, DNA fragments or RNA fragments are used accordingly in response to the analysis method employed. The base length of nucleic acids for analyzing polymorphism may be sufficient if it exerts respective functions of each nucleic acid. Base lengths in the case of use as primers are, for example, 10 bp to 50 bp, preferably 15 bp to 40 bp, and more preferably 15 bp to 30 bp.

Note here that in the case of use as primers, some mismatches to the sequence

which constitutes the template may be admitted as long as the primer can specifically hybridize the subject for amplification and amplify the target DNA fragment. In the case of probes, some mismatches to the sequence which is subject to detection may be similarly admitted as long as the probe can specifically hybridize the sequence which is subject to detection. The numbers of mismatches are one to several, preferably one to five, and more preferably one to three.

Nucleic acids (primers and probes) for analyzing polymorphism can be synthesized in accordance with known methods, e.g., phosphodiester method. Note here that textbooks (e.g., Molecular Cloning, Third Edition, Cold Spring Harbor Laboratory Press, New York) can be referred with respect to design, synthesis, and others of nucleic acids for analyzing polymorphism.

Nucleic acids for analyzing polymorphism in the present invention can be labeled with labeling substances in advance. The use of such labeled nucleic acids allows, for example, the analysis of polymorphism by using the labeling amount in the product of amplification as a marker. Furthermore, by labeling two kinds of primers which were designed specifically amplify the partial DNA region in the gene of each genotype that constitute polymorphism with labeling substances that are different from each other, the genotype in a nucleic acid sample can be discriminated according to the labeling substance and labeling amount to be detected based on the product of amplification. Concrete examples of detection methods using these labeled primers may include: a method for detecting polymorphism, which includes labeling, with fluorescein isothiocyanate and Texas red, two kinds of nucleic acid primers (allele-specific sense primers) that respectively and specifically hybridize the sense strand of each genotype constituting polymorphism; amplifying the partial DNA region including the site of polymorphism by using these labeled primers and the antisense primers that specifically hybridize the antisense strand; and measuring the labeling amount of each fluorescent substance in the product of amplification obtained. Note here that labeling of the antisense primer herein with, for example, biotin allows the separation of the product of amplification by utilizing the specific binding between biotin and avidin.

Radioactive isotopes, for example, ^{32}P, and fluorescent substance, for example, fluorescein isothiocyanate, tetramethylrhodamine isothiocyanate, and Texas red, etc. can be exemplified as labeling substances to be used in labeling nucleic acids for analyzing polymorphism. The 5' terminal labeling method using alkaline phosphatase and T4 polynucleotide kinase, the 3' terminal labeling method using T4 DNA polymerase and Klenow fragment, nicktranslation method, random primer method (Molecular Cloning, Third Edition, Chapter 9, Cold Spring Harbor Laboratory Press, New York), and the like can be exemplified as labeling methods.

The above-mentioned nucleic acids for analyzing polymorphism can be used also under a condition fixed to an insoluble support. Processing of an insoluble support to be used for the fixation to several forms such as chips and beads allows the more simplified analysis of polymorphism by using these fixed nucleic acids.

A nucleic acid sample can be prepared from blood, skin cells, mucous cells, hair, and others from the subject according to known extraction methods and purification methods. In the case of including the gene which is subject to the analysis of polymorphism, the genome DNA of arbitrary length can be used as a nucleic acid sample. Furthermore, it is not necessary to use a nucleic acid sample in which all genes subject to the analysis are present on one nucleic acid. That is to say, as a nucleic acid sample of the present invention, both material in which all genes subject to the analysis are present on one nucleic acid and material in which genes subject to the analysis are present separately on two or more nucleic acids can be used. Note here that material in a fragmented or partial condition may be accepted as long as the site of polymorphism to be analyzed is at least present, although genes subject to the analysis in a nucleic acid sample are not in a complete condition (i.e., a condition in which the full length of the gene is present).

Analysis of each gene polymorphism is carried out each by each of the gene polymorphism, or a plurality or entire gene polymorphisms are carried out simultaneously. In the former case, for example, nucleic acid sample collected from the subjects is divided in accordance with the number of polymorphisms to be analyzed, and analysis of polymorphism is carried out individually. In the latter case, for example, analysis of polymorphism can be carried out by DNA chip or micro-array. Note here that "simultaneously" herein not only imply that all operations of the analysis process are conducted simultaneously but also include the case in which part of operations (e.g., operation to amplify nucleic acid, hybridization or detection of the probe) is conducted simultaneously.

Polymorphism of each gene can be analyzed by using mRNA which is a product of transcription of the gene which is subject to the analysis. After extracting and purifying mRNA of the gene, which is subject to the analysis, from blood, urine, and others of the subject, for example, polymorphism can be analyzed with mRNA as a starting material by conducting methods, e.g., Northern blotting method (Molecular Cloning, Third Edition, 7.42, Cold Spring Harbor Laboratory Press, New York), dot blotting method (Molecular Cloning, Third Edition, 7.46, Cold Spring Harbor Laboratory Press, New York), RT-PCR method (Molecular Cloning, Third Edition, 8.46, Cold Spring Harbor Laboratory Press, New York), and methods using the DNA chip (DNA array), and the like.

In addition, in the above-mentioned polymorphism, polymorphism involved with changes in amino acids can be analyzed by using the expression product of gene that is a subject to analysis. In this case, material, even if it is partial protein or partial peptide, can be used as a sample for analysis as long as it contains amino acids which correspond to the site of polymorphism.

Analysis methods using these expression products of gene may include: a method for directly analyzing amino acids at the site of polymorphism, a method for immunologically analyzing utilizing changes of three-dimensional structure, or the like. As the former method, a well-known amino acid sequence analysis method (a method using Edman method) can be used. As the latter method, ELISA (enzyme-linked immunosorbent assay) using a monoclonal antibody or polyclonal antibody which has binding activity specific to the expression product of gene which has any of genotypes that constitute polymorphism; radioimmunoassay, immunoprecipitation method, immunodiffusion method, and the like, can be used.

Information about polymorphisms to be obtained by conducting the detection methods of the present invention described above can be used to diagnose a genetic risk for hypertension. That is to say, the present invention also provides a method for diagnosing a genetic risk for hypertension, which includes a step of determining the genotype in a nucleic acid sample based on information about polymorphisms obtained by the above-detection methods, and a step of assessing a genetic risk for hypertension based on the determined genotype in the nucleic acid sample. Herein, the determination of the genotype is typically to determine which genotype both alleles of nucleic acid samples have with respect to the polymorphism to be detected. In the case where the subject to be detected is, for example, GPIa (1648A→G) polymorphism, the detection of genotype is typically, an investigation on what genotype from AA (the base at position 1648 is a homozygote of allele A), AG (the base at position 1648 is a heterozygote of allele A and allele G) and GG (the base at position 1648 is a homozygote of allele G), the GPIa gene has in a nucleic acid sample.

By considering the results obtained in Example mentioned below, in order to enable a diagnosis of genetic risk for hypertension with high accuracy and high predictability, for example, in the case of the GPIa (1648A→G) polymorphism, it is determined whether the genotype in a nucleic acid sample is GG or, AA or AG. Similarly, in the case of the CCR2 (190G→A) polymorphism, it is determined whether the genotype is AA, or GG or GA; in the case of the ApoC-III (1100C→T) polymorphism, it is determined whether the genotype is TT, or CC or CT; in the case of the GPß3 (825C→T) polymorphism, it is determined whether the genotype is CT or

TT, or CC; in the case of the TNFa (-850C→T) polymorphism, it is determined whether the genotype is TT, or CC or CT; in the case of TNFa (-238G→A) polymorphism, it is determined whether the genotype is GA or AA, or GG; in the case of IRS-1 (3494G→A) polymorphism, it is determined whether the genotype is GA or AA, or GG; and in the case of GPIba (1018C→T) polymorphism, it is determined whether the genotype is CT or TT, or CC.

Diagnosis of a genetic risk for hypertension enables prediction of potentiality (likelihood of development) in that hypertension might be developed in future, that it to say, development risk (susceptibility to development). Furthermore, it becomes possible to carry out the recognition of hypertension based on the genotype that is an objective index or to understand conditions of the disease. In other words, the diagnosis method of the present invention makes it possible to evaluate the risk of development of hypertension, to recognize the development of hypertension, or to understand conditions of the disease. It is clinically significant that it is possible to assess the risk of development because having knowledge about the development risk in advance contributes to primary prevention of hypertension so as to makes it possible to take an appropriate prevention.

The information obtained by the diagnosis method of the present invention can be used for selecting an appropriate treatment, improvement of prognosis, improvement of QOL (quality of life) of patients, reduction of the risk of development, or the like.

By conducting the diagnosis method of the present invention, it is possible to monitor the development risk for hypertension, etc. As a result of such monitoring, when correlation between certain external factors (environment factor, administration of drugs, and the like) and the increase in the risk of development is found, the relevant external factors are recognized as risk factors and it can be thought that based on such information, the development risk etc. can be reduced.

The genetic information associated with the development of hypertension obtained by the present invention can be used for treatment of hypertension (including preventive treatment). For example, as a result of carrying out the diagnostic method of the present invention, when the polymorphism to be analyzed is a genotype to increase the risk of development of hypertension, by introducing a gene having a genotype with low risk of development into a living body and allowing the gene to express, the reduction of disease, suppression of development and reduction of development risk, and the like can be expected due to the expression of the gene. The same treatment effect can be expected by a method of introducing antisense strand with respect to mRNA of gene having a genotype with high risk of development and

suppressing the expression of the mRNA.

The introduction of genes or antisense strand can be carried out by a method, for example, a method using a plasmid for gene introduction or a virus vector, electroporation (Potter, H. et al., Proc. Natl. Acad. Sci. U.S.A. 81, 7161-7165(1984), an ultrasonic micro bubble (Lawrie, A., et al. Gene Therapy 7, 2023-2027 (2000)), lipofection (Felgner, P.L. et al., Proc. Natl. Acad. Sci. U.S.A. 84, 7413-7417 (1984)), microinjection (Graessmann, M. & Graessmann, A., Proc. Natl. Acad. Sci. U.S.A. 73,366-370(1976)), and the like. By utilizing these methods, desired genes, etc. can be directly introduced (in vivo method) or indirectly introduced (ex vivo method).

The second aspect of the present invention provides a kit (a kit for detecting the genotype or a kit for diagnosing hypertension) to be used in the above-mentioned detecting method or diagnostic method in the present invention. Such a kit contains nucleic acids (nucleic acids for analyzing polymorphism) for analyzing two or more polymorphisms selected from the group consisting of polymorphisms described in (1) to (4) above. As another embodiment, such a kit is constructed, which contains nucleic acids (nucleic acids for analyzing polymorphism) for analyzing two or more polymorphisms selected from the group consisting of polymorphisms described in (5) to (8) above.

Nucleic acids for analyzing polymorphism are designed as materials which can specifically amplifies (primer) or specifically detect (probe) the DNA region containing the polymorphism portion to be analyzed or mRNA which corresponds to the region in the analysis methods to be applied (a method which utilizes PCR using the above-mentioned allele-specific nucleic acids and the like, PCR-RFLP method, PCR-SSCP method, TaqMan-PCR method, Invader method, etc.). Concrete examples of kits to be provided according to the present invention are described below.

A kit for detecting the genotype, comprising two or more nucleic acids selected from the group consisting of the following (1) to (4):

(1) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose base at position 1648 is A, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose base at position 1648 is G;

(2) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene whose base at position 190 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene whose base at position 190 is A;

(3) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1100 of the apolipoproteins C-III gene whose base at position 1100 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1100 of the apolipoproteins C-III gene whose base at position 1100 is T; and

(4) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 825 of the G-protein ß3 subunit gene whose base at position 825 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -482 of the G-protein ß3 subunit gene whose base at position 825 is T.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (1) to (4). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (1) to (4) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (2) to (4) (nucleic acids for analyzing polymorphisms with three highest odds ratio).

A kit for detecting the genotype, comprising two or more nucleic acids selected from the group consisting of the following (5) to (8):

(5) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene whose base at position -850 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene whose base at position -850 is T;

(6) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene whose base at position -238 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene whose base at position -238 is A;

(7) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene whose base at position 3494 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene whose base at position 3494 is A; and

(8) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene whose base at position 1018 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene whose base at position 1018 is T.

In the above mention, kits are constructed by selecting two or more nucleic

acids from the group consisting of (5) to (8). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (5) to (8) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (5), (7) and (8) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (1) to (4):

(1) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene only in the case where the base at position 1648 of the glycoprotein Ia gene in a nucleic acid sample is A, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene only in the case where the base at position 1648 of the glycoprotein Ia gene in a nucleic acid sample is G;

(2) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene only in the case where the base at position 190 of the chemokine receptor 2 gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene only in the case where the base at position 190 of the chemokine receptor 2 gene in a nucleic acid sample is A;

(3) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1100 of the apolipoprotein C-III gene only in the case where the base at position 1100 of the apolipoprotein C-III gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1100 of the apolipoprotein C-III gene only in the case where the base at position 1100 of the apolipoprotein C-III gene in a nucleic acid sample is T; and

(4) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 825 of the G-protein ß3 subunit gene only in the case where the base at position 825 of the G-protein ß3 subunit gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 825 of the G-protein ß3 subunit gene only in the case where the base at position 825 of the G-protein ß3 subunit gene in a nucleic acid sample is T.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (1) to (4). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (1) to

(4) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (2) to (4) (nucleic acids for analyzing polymorphisms with three highest odds ratio in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (5) to (8):

(5) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene only in the case where the base at position -850 of the tumor necrosis factor-α gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene only in the case where the base at position -850 of the tumor necrosis factor-α gene in a nucleic acid sample is T;

(6) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene only in the case where the base at position -238 of the tumor necrosis factor-α gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene only in the case where the base at position -238 of the tumor necrosis factor-α gene in a nucleic acid sample is A;

(7) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene only in the case where the base at position 3494 of the insulin receptor substrate-1 gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene only in the case where the base at position 3494 of the insulin receptor substrate-1 gene in a nucleic acid sample is A; and

(8) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene only in the case where the base at position 1018 of the glycoprotein Iba gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene only in the case where the base at position 1018 of the glycoprotein Iba gene in a nucleic acid sample is T.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (5) to (8). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (5) to (8) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (5), (7) and (8) (nucleic acids for analyzing polymorphisms with three

highest odds ratio in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (1) to (4):

(1) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose base at position 1648 is A and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose gene at position 1648 is G and of an antisense primer that specifically hybridizes a partial region of the glycoprotein Ia gene;

(2) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene and which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene whose base at position 190 is G and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position 190 of the chemokine receptor 2 gene whose gene at position 190 is A and of a sense primer that specifically hybridizes a partial region of the chemokine receptor 2 gene;

(3) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1100 of the apolipoprotein C-III gene; and

(4) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene whose base at position 825 is C and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene whose gene at position 825 is T and of an antisense primer that specifically hybridizes a partial region of the G-protein β3 subunit gene.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (1) to (4). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (1) to (4) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (2) to (4) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (5) to (8):

(5) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene and which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene whose base at position -850 is C and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position -850 of the tumor necrosis factor-α gene whose gene at position -850 is T and of a sense primer that specifically hybridizes a partial region of the tumor necrosis factor-α gene;

(6) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene and which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene whose base at position -238 is G and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position -238 of the tumor necrosis factor-α gene whose gene at position -238 is A and of a sense primer that specifically hybridizes a partial region of the tumor necrosis factor-α gene;

(7) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene whose base at position 3494 is G and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene whose gene at position 3494 is A and of an antisense primer that specifically hybridizes a partial region of the insulin receptor substrate-1 gene; and

(8) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene whose base at position 1018 is C and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene whose gene at position 1018 is T and of an antisense primer that specifically hybridizes a partial region of the glycoprotein Iba gene.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (5) to (8). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (5) to (8) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (5), (7) to (8) (nucleic acids for analyzing polymorphisms with three highest odds ratio in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (1) to (4);

(1) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 1648 in the antisense strand of the glycoprotein Ia gene whose base at position 1648 is A and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 1648 in the antisense strand of the glycoprotein Ia gene whose base at position 1648 is G and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the glycoprotein Ia gene and that can specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene in concurrent use with the above first or second nucleic acid;

(2) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 190 in the sense strand of the chemokine receptor 2 gene whose base at position 190 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 190 in the sense strand of the chemokine receptor 2 gene whose base at position 190 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the chemokine receptor 2 gene and that can specifically amplify the partial DNA region containing the base at position 190 of the chemokine receptor 2 in concurrent use with the above first or second nucleic acid;

(3) a set of nucleic acids which consists of first and second nucleic acids that are designed to specifically amplify the partial DNA region containing the base at position 1100 of the apolipoprotein C-III gene, of a third nucleic acid that specifically hybridizes the nucleic acid which is amplified by the use of the first and second nucleic acids using the apolipoprotein C-III gene in which the base at position 1100 is C as a template and which is labeled with a first labeling substance, and of a fourth nucleic acid that specifically hybridizes a nucleic acid which is amplified by the use of the first and second nucleic acids by using apolipoprotein C-III gene in which the base at position 1100 is T as a template and which is labeled with a second labeling substance; and

(4) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 825 in the antisense strand of the G-protein ß3 subunit gene whose base at position 825 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 825 in the antisense strand of the G-protein ß3 subunit gene whose base at position 825 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in

the sense strand of the G-protein ß3 subunit gene and that can specifically hybridize the partial DNA region containing the base at position 825 of the G-protein ß3 subunit gene in concurrent use with the above first or second nucleic acid.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (1) to (4). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (1) to (4) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (2) to (4) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (5) to (8);

(5) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position -850 in the sense strand of the tumor necrosis factor α gene whose base at position -850 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position -850 in the sense strand of the tumor necrosis factor α gene whose base at position -850 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the tumor necrosis factor α gene and that can specifically amplify the partial DNA region containing the base at position -850 of the tumor necrosis factor α in concurrent use with the above first or second nucleic acid;

(6) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position -238 in the sense strand of the tumor necrosis factor α gene whose base at position -238 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position -238 in the sense strand of the tumor necrosis factor α gene whose base at position -238 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the tumor necrosis factor α gene and that can specifically amplify the partial DNA region containing the base at position -238 of the tumor necrosis factor α in concurrent use with the above first or second nucleic acid;

(7) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 3494 in the antisense strand of the insulin receptor substrate-1 gene whose base at position 3494 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 3494 in the antisense strand of the insulin receptor substrate-1 gene whose base at position 3494 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the insulin receptor substrate-1 gene

and that can specifically amplify the partial DNA region containing the base at position 3494 of the insulin receptor substrate-1 gene in concurrent use with the above first or second nucleic acid; and

(8) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 1018 in the antisense strand of the glycoprotein Iba gene whose base at position 1018 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 1018 in the antisense strand of the glycoprotein Iba gene whose base at position 1018 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the glycoprotein Iba gene and that can specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Iba gene in concurrent use with the above first or second nucleic acid.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (5) to (8). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (5) to (8) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (5), (7) to (8) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

In the above-mentioned kits, one or two or more of reagents (buffer, reagent for reaction, and reagent for detection, etc.) may be combined in response to the usage of the kit.

The present invention is hereinafter described in more detail by way of Examples.

[Example 1] Selection of gene polymorphism

By using several kinds of public databases including PubMed [National Center for Biological Information (NCBI)], Online Mendelian inheritance in Men (NCBI), Single Nucleotide Polymorphism (NCBI), etc., from a comprehensive viewpoint including vascular biology, platelet-leukocyte biology, coagulation and fibrinolysis system, a metabolic factor such as lipid, sugar, etc., 71 genes which were estimated to be associated with coronary arteriosclerosis, coronary artery spasm, hypertension, diabetes mellitus, hyperlipidemia, etc. were extracted from genes which had been previously reported. Furthermore, among the polymorphisms existing in these genes, 112 polymorphisms including polymorphisms which exist in promoter regions or exons, or polymorphisms which were located in splice donor sites or acceptor sites and expected to be associated with the functional changes of gene products were selected (Figures 1 and 2).

[Example 2] Determination of gene polymorphism

The study population comprised 1940 Japanese individuals (1107 men and 833 women) who either visited outpatient clinics of or were admitted to one of the 15 participating hospitals between July 1994 and December 2001. A total of 1067 subjects (574 men and 493 women) either had hypertension [systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥ 90 mmHg, or both] or had taken antihypertensive drugs. Cases with coronary artery disease, valvular heart disease, congenital malformations of the heart or vessels, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the study. The 873 control subjects (533 men and 340 women) with normal blood pressure (systolic blood pressure of < 140 mmHg and diastolic blood pressure of < 90 mmHg) were recruited from individuals who were found to have at least one of the conventional risk factors for coronary artery disease, including habitual cigarette smoking (≥10 cigarettes daily), obesity [body mass index of > 26 kg/m^2], diabetes mellitus (fasting blood glucose of > 126 mg/dL or hemoglobin A_{1c} of > 6.5%, or both), hypercholesterolemia (serum total cholesterol of > 220 mg/dL), and hyperuricemia (serum uric acid of > 7.7 mg/dL for men or > 5.5 mg/dL for women), but who had no history of coronary artery disease. These controls showed normal resting electrocardiogram, and also in exercise tolerance test, no myocardial ischemic change was shown. Blood pressure was measured with subjects in the seated position according to the guidelines of the American Heart Association (Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ. Human blood pressure determination by sphygmomanometry. Circulation. 1993;88: 2640-2470.).

From each of the subjects, 7 mL of venous blood was collected in a tube containing 50 mmol/L EDTA-2Na and genome DNA was extracted by using a DNA extraction kit (Qiagen, Chatsworth, CA). Genotypes of single nucleotide polymorphisms were determined with a fluorescence- or colorimetry-based allele-specific primer-probe assay system (Toyobo Gene Analysis, Tsuruga, Japan) (see Figure 3). DNA fragment containing a polymorphism site was amplified by polymerase chain reaction (PCR) by using two kinds of allele specific sense primers (or antisense primers) whose 5' end were labeled with fluorescein isothiocyanate (FITC) or Texas red (TxR) and an antisense primer (or a sense primer) whose 5' end was labeled with biotin. Alternatively, DNA fragment containing polymorphism site was amplified by PCR by using two kinds of allele specific sense (or antisense) primers and an antisense (or a sense) primer whose 5' end was labeled with biotin, or by using a sense primer and an antisense primer whose 5' end was labeled with biotin. The reaction solution (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 1 to 4 mmol/L of $MgCl_2$, 1 U of DNA

polymerase (rTaq or KODplus; Toyobo Co., Ltd. Osaka, Japan) in corresponding DNA polymerase buffer. The amplification protocol comprised an initial denaturation at 95°C for 5 minutes; 35 to 45 cycles of denaturation at 95°C for 30 seconds, annealing at 55 to 67.5°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 2 minutes.

For determination of genotype by fluorescence, amplified DNA was incubated with a solution containing streptavidin-conjugated magnetic beads in 96-well plates at room temperature. The plates were placed on a magnetic stand, supernatants were collected from the wells and then transferred to the wells of a 96-well plate containing 0.01 M NaOH, followed by measuring fluorescence by microplate reader at excitation wavelength and fluorescence wavelength of 485 nm and 538 nm for FITC and at excitation wavelength and fluorescence wavelength of 584 nm and 612 nm for TxR. Furthermore, for determination of genotype by colorimetry, amplified DNA was denatured with 0.3 M NaOH and then subjected to hybridization at 37°C for 30 min in hybridization buffer containing 35 to 40% formamide with any of allele-specific capture probes fixed to the bottom of the wells of a 96-well plate. After thorough washing of the wells, alkaline phosphatase–conjugated streptavidin was added to each well and the plate was shaken at 37°C for 15 min. The wells were again washed, and, after the addition of a solution containing 0.8 mM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (monosodium salt) and 0.4 mM 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt, the absorbance at 450 nm was measured.

To confirm the accuracy of genotyping by this method, DNA samples of 50 people were selected at random, and the samples were subjected to PCR-restriction fragment length polymorphism method or direct sequencing method of PCR products. In any samples, the genotype determined by the allele specific primer-probe measurement system was identical to that determined by PCR-restriction fragment length polymorphism method or direct sequencing method.

Statistical analysis in the following association study was carried out as follows. Quantitative clinical data were compared between patients with hypertension and controls by the unpaired Student's *t* test or the Mann-Whitney *U* test. Qualitative data were compared by the chi-square test. Allele frequencies were estimated by the gene counting method, and the chi-square test was used to identify significant departures from Hardy-Weinberg equilibrium. The present inventors performed multivariate logistic regression analysis to adjust risk factors, with hypertension as a dependent variable and independent variables including age, body mass index (BMI), smoking status (0 = nonsmoker, and 1 = smoker), metabolic variables (0 = no history of diabetes mellitus, hypercholesterolemia, or hyperuricemia;

and 1 = positive history), and genotype of each polymorphism. Each genotype was assessed according to dominant, recessive, and additive genetic models, and the *P* value, odds ratio, and 95% confidence interval were calculated. For combined genotype analyses, the present inventors performed the stepwise forward selection method of multivariate logistic regression to calculate odds ratios for each combined genotype.

[Example 3] Selection of polymorphism associated with hypertension and development of method for diagnosing hypertension

The present inventors performed an association study of the 112 polymorphisms of the 71 candidate genes with myocardial infarction in 451 men (myocardial infarction: 219, control: 232) and in 458 women (myocardial infarction: 226, control: 232) in the previous report (Yamada Y, Izawa H, Ichihara S, et al. Prediction of the risk of myocardial infarction from polymorphisms in candate genes. N Engl J Med. in press). In this study, the present inventors have found that 19 and 18 single nucleotide polymorphisms were associated with the development of myocardial infarction in men and women, respectively, which included candidate genes of hypertension (see Figures 1, 2 and 4). In this Example, a large scale association study on the association of these single nucleotide polymorphisms with hypertension was carried out in total 1940 cases.

The background data of all 1940 participants (1107 men and 833 women) are shown in Figure 5. For men, age, BMI, the prevalence of hyperuricemia, and the serum concentration of creatinine as well as systolic and diastolic blood pressure were significantly greater, and the prevalence of smoking was significantly lower, in subjects with hypertension than in controls. For women, age, BMI, and the prevalence of hypercholesterolemia and hyperuricemia as well as systolic and diastolic blood pressure were significantly greater in subjects with hypertension than in controls.

Multivariate logistic regression analysis with adjustment for age, BMI, and the prevalence of smoking, diabetes mellitus, hypercholesterolemia, and hyperuricemia revealed that 4 of the 19 polymorphisms examined for men and 4 of the 18 polymorphisms examined for women were significantly associated with hypertension ($P < 0.05$ in either a dominant or recessive genetic model) (see Figure 6). The genotype distributions of these polymorphisms are shown (see Figure 7).

The present inventors performed the stepwise forward selection method of multivariate logistic regression analysis (see Figure 8) with either a dominant or recessive model for each polymorphism based on the *P* value (the lower *P* value) for

association with hypertension shown in Figure 6. The chromosomal loci of the corresponding genes are also shown in Figure 8.

The –850C→T and –238G→A polymorphisms of the tumor necrosis factor–α gene were not in linkage disequilibrium [pairwise linkage disequilibrium coefficient, D' (D/D_{max}), of –0.310 and standardized linkage disequilibrium coefficient, r, of –0.020; $P = 0.613$, chi-square test]. Odds ratios for susceptibility to hypertension based on combined genotypes with the stepwise forward selection method for men and women separately are shown in Figure 9 and 11 (A) and in Figure 10 and 11(B), respectively. For men, combined genotype analysis of the four polymorphisms (GPIa (1648A→G) polymorphism, CCR2 (190G→A) polymorphism, ApoC-III (1100C→T) polymorphism, GPß3 (825C→T) polymorphism) revealed that the maximal odds ratio was 5.34 (Figure 9 and 11(A)). For women, combined genotype analysis of the four polymorphisms (TNFa (-850C→T) polymorphism, TNFa (-238G→A) polymorphism, IRS-1 (3494G→A) polymorphism, GPIba (1018C→T) polymorphism) revealed that the maximal odds ratio was 46.86 (Figures 10 and 11(B)).

As mentioned above, multivariate logistic regression analysis revealed that four SNPs related to hypertension in men and women, respectively. That is to say, the relation of hypertension to 19 SNPs for men and 18 SNPs for women was examined in a large-scale association study with 1940 individuals, and four each of the polymorphism related to hypertension development in men and women were identified. Furthermore, the present inventors developed a genetic risk diagnosis system for hypertension based on the combined genotypes for these SNPs that yielded maximal odds ratios of 5.34 for men and 46.86 for women by the stepwise forward selection method of multivariate logistic regression analysis.

The regulation of blood pressure involves both the integration of a variety of biological systems that control the structure and tone of the vasculature and the volume and composition of body fluid, as well as the adaptation of these systems to constantly changing physiological needs (Lalouel J-M, Rohrwasser A. Development of genetic hypotheses in essential hypertension. J Hum Genet. 2001;46: 299-306). The relation of hypertension to 19 SNPs for men and 18 SNPs for women examined for men and women, respectively, in the present study were selected on the basis of a comprehensive overview of vascular biology, platelet-leukocyte biology, the fibrinolysis system, as well as lipid and glucose metabolism and other metabolic factors. Actually, the genes now shown to be associated with hypertension may play roles in diverse aspects of the etiology of this condition, including vascular biology (G protein β3 subunit), inflammation (tumor necrosis factor–α), monocyte and

lymphocyte biology (chemokine receptor 2), platelet function (glycoproteins Ia and Ibα), lipid metabolism (apolipoprotein C-III), and insulin and glucose metabolism (insulin receptor substrate–1). The maximal odds ratios obtained with the genetic risk diagnosis system for hypertension developed by the present inventors (5.34 for men and 46.86 for women) appear to be the highest such values reported by large-scale association studies of hypertension particularly in women. Among the eight polymorphisms associated with hypertension, the –850C→T and –238G→A polymorphisms of the tumor necrosis factor-α gene yielded the highest odds ratio for predisposition to hypertension in women. The tumor necrosis factor–α gene locus was previously shown to be associated with obesity-related hypertension in French Canadian (Pausova Z, Deslauriers B, Gaudet D, Tremblay J, Kotchen TA, Larochelle P, Cowley AW, Hamet P. Role of tumor necrosis factor-a gene locus in obesity and obesity-associated hypertension in French Canadians. Hypertension. 2000;36: 14-19). Furthermore, a –308A→G polymorphism of the tumor necrosis factor–α gene also previously showed a tendency to associate with essential hypertension, although statistical significance was not achieved (Frossard PM, Gupta A, Pravica V, Perry C, Hutchinson IV, Lukic ML. A study of five human cytokine genes in human essential hypertension. Mol Immunol. 2002;38: 969-976.). The serum concentration of this the tumor necrosis factor–α was associated with systolic blood pressure and insulin resistance in a native Canadian population (Zinman B, Hanley AJG, Harris SB, Kwan J, Fantus IG. Circulating tumor necrosis factor-a concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocrinol Metab. 1999;84: 272-278.). Tumor necrosis factor–α stimulates the production of the potent vasoconstrictor endothelin-1 (Kahaleh MB, Fan PS. Effect of cytokines on the production of endothelin by endothelial cells. Clin Exp Rheumatol. 1997;15: 163-167.) and the serum concentrations of these two agents were positively correlated in subjects with obesity (Winkler G, Lakatos P, Salamon F, Nagy Z, Speer G, Kovacs M, Harmos G, Dworak O, Cseh K. Elevated serum TNF-a level as a link between endothelial dysfunction and insulin resistance in normotensive obese patients. Diabetes Med. 1999;16: 207-211.). These findings and the above-mentioned results by the present inventors suggest that the tumor necrosis factor–α gene is a candidate locus for susceptibility to hypertension. With regard to the other six polymorphisms associated with hypertension in the present study, the 825C→T polymorphism of the G protein β3 subunit gene was previously associated with hypertension (Siffert W, Rosskop D, Siffert G, Busch S, Moritz A, Erbel R, Sharma AM, Ritz E, Wichmann H-E, Jakobs KH, Horsthemke B. Association of a human G-protein ß3 subunit variant with hypertension. Nat Genet. 1998;18: 45-48.), and a polymorphism of the apolipoprotein C-III gene was also reported to be associated with blood pressure (Tas S, Abdella NA. Blood pressure, coronary artery disease, and glycaemic control in type 2 diabetes mellitus: relation to apolipoprotein-CIII gene polymorphism. Lancet. 1994;343: 1994-1995.). Chemokine

receptor 2 and insulin receptor substrate–1 genes have been shown to contribute to the development of hypertension (Bush E, Maeda N, Kuziel WA, Dawson TC, Wilcox JN, DeLeon H, Taylor WR. CC chemokine receptor 2 is required for macrophage infiltration and vascular hypertrophy in angiotensin II-induced hypertension. Hypertension. 2000;36: 360-363., Abe H, Yamada N, Kamata K, Kuwaki T, Shimada M, Osuga J, Shionoiri F, Yahagi N, Kadowaki T, Tamemoto H, Ishibashi S, Yazaki Y, Makuuchi M. Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. J Clin Invest. 1998;101: 1784-1788.). Furthermore, platelet activation may be important in the etiology of essential hypertension (Andrioli G, Ortolani R, Fontana L, Gaino S, Bellavite P, Lechi C, Minuz P, Manzato F, Tridente G, Lechi A. Study of platelet adhesion in patients with uncomplicated hypertension. J Hypertens. 1996;14: 1215-1221., Dockrell ME, Walker BR, Noon JP, Watt GC, Williams BC, Webb DJ. Platelet aggregation in young men contrasting predisposition to high blood pressure. Am J Hypertens. 1999;12: 115-119., Bereczki C, Tur S, Nemeth I, Sallai E, Torday C, Nagy E, Haszon I, Papp F. The roles of platelet function, thromboxane, blood lipids, and nitric oxide in hypertension of children and adolescents. Prostaglandins Leukot Essent Fatty Acids. 2000;62: 293-297.); although the polymorphisms in the glycoprotein Ia and glycoprotein Ibα genes studied here have been associated with coronary artery disease (Kroll H, Gardemann A, Fechter A, Haberbosch W, Santoso S. The impact of the glycoprotein Ia collagen receptor subunit A1648G gene polymorphism on coronary artery disease and acute myocardial infarction. Thromb Haemost. 2000;83: 392-396., Murata M, Matsubara Y, Kawano K, et al. Coronary artery disease and polymorphisms in a receptor mediating shear stress-dependent platelet activation. Circulation. 1997;96: 3281-6.), they have not previously been associated with hypertension.

It is possible that some of the polymorphisms examined in the present Examples are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of hypertension. The present results indicate, however, that glycoprotein Ia, chemokine receptor 2, apolipoprotein C-III, and the G protein β3 subunit are susceptibility loci for hypertension in Japanese men, and that tumor necrosis factor–α, insulin receptor substrate–1, and glycoprotein Ibα constitute such loci in Japanese women. Moreover, the combined genotypes for these polymorphisms may prove informative for determination of the genetic risk for hypertension. The genetic diagnosis system by the present inventors should therefore contribute to the primary prevention of hypertension and of cardiovascular diseases, stroke, or renal diseases induced by this condition.

The present invention is not limited to the description of the above

embodiments. A variety of modifications, which are within the scopes of the following claims and which are achieved easily by a person skilled in the art, are included in the present invention.

INDUSTRIAL APPLICABILITY

According to the present invention, gene polymorphisms associated with hypertension are analyzed and the genotype in a nucleic acid sample is detected. By using the information about the polymorphisms obtained by the detection of the genotype, diagnosis of the risk for hypertension with high accuracy and high predictability can be carried out. Therefore, the present invention provides an effective means for understanding in advance the risk of development of hypertension, so that it can be expected that the means should therefore contribute to the primary prevention of hypertension as well as to contribute the prevention of cardiovascular diseases, stroke, renal diseases, or the like, induced by hypertension. Furthermore, according to the present invention, auxiliary information useful for the diagnosis of hypertension can be obtained so as to enable an appropriate treatment and therefore prognosis can be improved. Furthermore, since the present invention provides useful information in elucidating the development mechanism of hypertension, it also provides an extremely important means for establishing a preventing method for hypertension.